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Enhancement of sporulation in species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* by growth on cellulose-containing substrates

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Abstract

Nine species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* were compared for sporulation on agar media and for enhancement of sporulation by growth on four cellulose-containing substrates (index card, filter paper, cheesecloth, cotton fabric). On two natural and one synthetic agar media, sporulation varied from profuse to nonexistent among three isolates of each species. Growth of all species on cellulose substrates resulted in large and significant increases in sporulation. Growth on index card pieces often provided the greatest increases, but no single substrate was superior for all species, and significant substrate × isolate interactions were observed within species. Overlay of filter paper onto whole colonies in agar plates resulted in 2 to 18-fold increases in sporulation for eight of nine species and production of spores in sufficient quantity for most experimental purposes. Overlay of soil dilution plates with filter paper to promote sporulation of colonies enabled detection of *B. spicifera*, *B. hawaiiensis*, *C. lunata*, and *E. rostratum* at relatively low population levels ($\leq 1.3 \times 10^3$ colony-forming units per gram of soil) in samples of a naturally infested soil. Results indicate that enhancement of sporulation by growth of species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* on cellulose substrates may facilitate (i) their identification in culture, (ii) production of spores at relatively high concentrations, and (iii) detection and enumeration of these fungi in soil.

Key words: *Bipolaris*, cellulose substrates, *Curvularia*, *Drechslera*, *Exserohilum*, sporulation

Introduction

Species of *Bipolaris*, *Drechslera*, *Exserohilum*, and *Curvularia* constitute a group of taxonomically related and ecologically similar deuteromycetes (mitosporic fungi) that are important plant pathogens or common saprophytes throughout the world. The first three of these genera were segregated from *Helminthosporium* in several revisions from 1930 to 1974, and some species of *Bipolaris* and *Curvularia* share the same teleomorph [1, 2]. As pathogens, species of the four genera are most important on a wide range of grasses, but many dicots also are infected [1–3]. Often multiple species occur together and infect host tissues simultaneously [4–7]. On most susceptible grasses, these pathogens primarily cause foliar lesions and blights [5, 8, 9], but infection also may extend into crowns, roots, stolons, and rhizomes to cause root rot, dieback, and melting out of stands [5, 8–10]. With *B. sorokiniana* (Sacc.) Shoemaker on small grains, initial infection and subsequent pathogenesis may occur entirely below ground [11, 14]. *B. sorokiniana*, *B. spicifera* (Banier) Subramanian, *C. geniculata* (Tracy & Earle) Boedijn, and *C. lunata* (Wakk.) Boedijn also have been isolated from agricultural and natural soils under diverse plant communities throughout the world [1], but

estimates of soil population levels have been presented only for *B. sorokiniana* [1, 13, 14].

Genera and species of dematiaceous hyphomycetes are identified largely on the basis of conidial morphology and hosts of origin [2, 9]. Conidial features used in taxonomy include size, shape, color, wall thickness, septation, and the presence of protruded hila in detached conidia. Specific morphological features for the practical identification of most species included in this study were recently summarized [7]. Molecular methods have been used to study relationships between isolates and races of some species within these four genera [e.g., 15], but such methods have not been used to identify species or evaluate taxonomic relationships.

Induction of sporulation by species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* is essential for their identification [2, 9]. In addition, large numbers of spores often are required for experimental purposes. As noted by Sivanesan [2], sporulation by species of these genera is usually abundant on naturally infected plant material, but some species may cease to sporulate after they are isolated and grown in pure culture. He recommended growing cultures on Sach's agar with sterilized plant material (commonly rice and barley grains, or leaves of maize and other plants) applied to the agar. Domsch et al. [1] and Couch [8] also listed agar media that have been used to obtain sporulation by individual species. Miles and Wilcoxson [16] obtained abundant sporulation by two species of *Bipolaris* and *Drechslera* on a mixture of agar and natural substrates, and Pratt [5] obtained satisfactory sporulation by species of *Bipolaris*, *Curvularia*, and *Exserohilum* on infested wheat and oat grain. With these few exceptions, however, the effectiveness of media, substrates, or techniques for inducing or enhancing sporulation have not been demonstrated across a range of species in these four genera.

Growth on cellulose substrates has been reported to enhance asexual sporulation by diverse imperfect fungi. Application of filter paper to the surfaces of agar media increased formation of pycnidia and conidia by *Pyrenochaeta* (*Phoma*) *terrestris* (E.M. Hans.) Gorenz, J.C. Walker, and R.H. Larson [17] and *Macrophomina phaseolina* (Tassi) Goid. [18]. Application of cellulose acetate membranes to agar surfaces increased conidium production by *Monilinia vaccinii-corymbosi* (Reade) Honey, but strong differences in isolate responses were observed [19]. Booth [20] recommended media containing carboxymethycellulose and pure cellulose lens paper for stimulating conidium formation by *Fusarium* spp. Similarly, growth of a nonsporulating fungus in a medium with cellulose as the sole carbon source induced sporulation and enabled its re-identification as a *Fusarium* sp. [21]. Although numerous cellulose-containing substrates have been used to obtain sporulation by species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* [1, 2, 5, 13], we are not aware of previous studies that have specifically evaluated enhancement of sporulation by growth on cellulose substrates for species of these genera.

In preliminary experiments, it was observed that for several species of *Bipolaris* and *Curvularia*, sporulation on pieces of paper index card placed on growing colonies was greatly increased over that present in the surrounding agar. It was not known whether such enhanced sporulation also occurs in other species of the four genera or when they are grown on other cellulose substrates. Therefore, this study was undertaken to fulfill four objectives: (i) to compare sporulation of nine species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* on agar media, (ii) to evaluate enhancement of sporulation by these fungi following growth on four cellulose substrates, (iii) to evaluate growth on cellulose substrates for production of large quantities of spores for experimental purposes; and (iv) to determine whether enhanced sporulation on a cellulose substrate can help to isolate and identify these fungi from soil. A preliminary report has been presented [22].

Materials and methods

Sources, storage, and growth of isolates

Three isolates each of *Bipolaris cynodontis* (Marig.) Shoemaker, *B. hawaiiensis* (Ellis) Uchida & Aragaki, *B. sorokiniana* (Sacc. Ex Sorok.) Shoemaker, *B. spicifera* (Banier) Subr., *B. stenospila* (Drechs.) Shoemaker, *Curvularia geniculata* (Tracy & Earle) Boedijn, *C. lunata* (Wakk.) Boedijn and *Exserohilum rostratum* (Drechs.) Leonard & Suggs were obtained by the author in recent years by direct transfer of individual spores from naturally infected leaves of bermudagrass

(*Cynodon dactylon* [L.] Pers.) in Mississippi as described [5, 6, 23]. Three isolates of *Drechslera dictyoides* (Drechs.) Shoemaker were obtained by the author in a similar manner from foliar lesions on annual ryegrass (*Lolium multiflorum* Lam.) in Mississippi in 2002. All isolates were stored in infested, dried wheat and oat grain at 10 °C [5], and kernels were plated on Difco cornmeal agar (CMA) to generate colonies. Growing colonies were maintained by serial transfers on CMA.

Sporulation on agar media

Isolates were transferred to edges of Difco potato dextrose agar (PDA), CMA, and Bilay's synthetic medium [20] in small (3.5 cm) plastic plates and grown for 10–14 days at 24–26 °C under plant growth lights [5] on a 12-h photoperiod. A single count of the number of spores present on the agar surface and in overlaying aerial mycelium in a 100× field of view then was made or attempted from the center of each plate. Sporulation by isolates of all species was compared on each agar medium in repeated experiments. In each experiment, plates were arranged in a randomized complete-block design with three replications during incubation. Each block consisted of 27 plates (1 per isolate) randomized in a transparent plastic bag. Mean numbers of spores observed per plate for 27 isolates of the 9 species were compared by analysis of variance except when conidia were too numerous to count.

Sporulation on cellulose substrates

Small plates of CMA were inoculated at edges with each isolate. A sterile piece of cellulose substrate or of CMA, each 5 × 5 mm, was placed in the center of each plate. Four cellulose substrates included in this study were: (1) pieces of paper index card (IC) (white, CID A-A–1611, Grade D) (NIB, Alexandria, VA 22311); (2) filter paper (FP) (Whatman No. 1) (Whatman House, Maidstone, Kent, UK ME16 OLS); (3) cheesecloth (CC) (4-layer, commercial grade no. 50, medium-weight, plain-weave, bleached cotton) (NIB, Alexandria, VA 22311); and (4) cotton fabric (CF) (preshrunk 100% white cotton T-shirt, 5.6 oz) (Alstyle Apparel and Activewear Manufacturing Co., Anaheim, CA 92805). Experiments were performed separately for each fungal species. In each experiment, three randomized complete blocks of fifteen plates (5 substrates × 3 isolates) were sealed in plastic bags and incubated under growth lights for 10 days. Each substrate piece then was removed to a 10 ml vial containing 0.25, 0.5, 1, 2, or 8 ml of 0.1% water agar (amount constant for each species) and agitated for 15 s with a microspatula to disperse spores. Substrate pieces were removed with a forceps from small quantities of suspension, or larger quantities were poured through double-layer cheesecloth, and 0.05 ml of spore suspension was deposited in a droplet on the surface of water agar. The total number of spores in one droplet from each suspension was counted at 100× after liquid was absorbed into agar.

For each fungal species, data were compared by ANOVA of a 3 (isolates) × 5 (substrates) factorial experiment, and sources of variation were evaluated for significance at $P = 0.05$. Significant differences between substrates and isolates were identified by use of Fisher's protected LSD test at $P = 0.05$.

Sporulation on CMA (+/−) filter paper

To evaluate production of large numbers of spores by the fungi on filter paper, sporulation on CMA in standard (9.5 cm diam) plates with and without overlaid filter paper was compared for each species. In each of two experiments, all three isolates of each species were transferred to the center of six plates, and after 24 h, sterile filter paper was applied to cover the agar surface in three of the plates. Plates were incubated under growth lights in randomized complete blocks (2 plates per block) for 2 weeks. Spores on the surface of each plate then were obtained in suspension by adding 5 or 10 ml of distilled water and scraping with a microspatula. Four hemacytometer counts were made of spore suspensions from each plate, and the mean concentration was used as a single replicate value in statistical analysis.

Assay for fungi in soil by sporulation on filter paper

To assay soil for presence of the nine fungal species as revealed by their sporulation on FP, 10 g oven-dried weight equivalent of a pasture soil was added to 90 ml distilled water, and additional 1/10 dilutions were prepared serially. From the

1×10^{-3}, 10^{-4}, and 10^{-5} dilutions, aliquots of 0.1 or 0.25 ml were streaked on CMA in standard plates. After 2 days, agar was inverted into large (15 cm diam) plates and bottoms were overlaid with sterile FP. Plates were incubated under growth lights for 10–14 days and observed for fungal sporulation on the FP.

Results

Sporulation on agar media

Sporulation on agar media varied from profuse to nonexistent among the nine species of dematiaceous hyphomycetes. On PDA, most isolates of *C. lunata* and *B. spicifera* formed conidia too numerous to count; isolates of *B. hawaiiensis*, *B. sorokiniana*, and *E. rostratum* formed few to numerous conidia; and isolates of *B. cynodontis*, *B. stenospila*, *C. geniculata*, and *D. dictyoides* formed few or none. However, conidia produced on PDA often were atypical (short, with few septations, often clavate or otherwise misshapen) in comparison to conidia formed on infected plants.

On CMA and Bilay's medium, most isolates of *C. lunata*, *C. geniculata*, *B. hawaiiensis*, *B. sorokiniana*, and *B. spicifera* produced moderate to numerous, normal-appearing conidia (often 100–2000 per ×100 microscopic field of view) while isolates of *B. cynodontis*, *B. stenospila*, *D. dictyoides*, and *E. rostratum* produced few or none. Significant differences in sporulation between isolates of the same species often were observed (data not presented).

Isolates of all species that sporulated on CMA and Bilay's medium often formed conidia in sectors (radial zones or clusters) rather than uniformly over the agar surface. Up to 10-fold or greater differences in numbers of conidia were observed between strongly and weakly sporulating sectors. Although species were not compared directly for sectoring of sporulation within colonies, this phenomenon usually was most common and conspicuous in *B. hawaiiensis*, *B. sorokiniana*, and *C. geniculata*, and less common or conspicuous in *C. lunata* and *B. spicifera*. Attempts to reduce or eliminate sectoring by repeated transfers of mycelium from sporulating areas of colonies were unsuccessful.

Sporulation on cellulose substrates

In repeated experiments to evaluate sporulation by three isolates of each fungal species on four cellulose substrates plus agar, significant ($P = 0.05$) heterogeneity of variance or experiment × treatment interactions were observed for seven of the nine species. Therefore, results of repeated experiments are described individually rather than combined.

In all 18 individual experiments (2 per fungal species), significant variation ($P = 0.05$ or 0.01) was attributed to substrates; this indicated that significant differences in sporulation on the different substrates (including agar) occurred across fungal isolates in both experiments. Significant variation due to isolates, which indicated significant differences between isolates in total sporulation across substrates, was observed in one experiment with *B. hawaiiensis*, *B. spicifera*, and *C. geniculata*, and in both experiments with *B. stenospila*. Significant isolate × substrate interactions, which indicated differences in patterns of sporulation on substrates by isolates within species, were observed in one experiment with *B. spicifera*, *B. sorokiniana*, *C. lunata*, and *E. rostratum*, and in both experiments with *B. stenospila*.

Results of 1 experiment for each fungal species are presented in Table 1. For seven species, results are presented from experiments in which significant isolate × substrate interactions did not occur; this enabled combining of results for the three isolates of each species. For *B. spicifera* and *B. stenospila*, significant ($P = 0.05$) isolate × substrate interactions occurred in both experiments; this required presenting results for individual isolates of these species.

Sporulation on one or more cellulose substrates was significantly greater than on agar in 11 of the 13 species or isolate comparisons (Table 1). Greatest sporulation was observed on IC in 10 of these instances and on CC in one instance.

In the second experiment with each fungal species, overall sporulation responses of species on the different cellulose substrates were generally similar to those observed in Experiment 1 (Table 1), but significant isolate × substrate interactions were observed for seven of the nine species (data not presented). These required comparisons of sporulation for individual rather than

Table 1. Mean numbers of spores produced by isolates of nine species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* following growth on four cellulose substrates and cornmeal agar

Fungal species	Isolates	Mean number of spores observed from four cellulose substrates and agar blocks[a]				
		IC	FP	CC	CF	Agar
C. lunata	Combined	72.8 ab[b]	69.4 ab	95.7 a	8.5 c	7.7 c
C. geniculata	Combined	36.6 a	13.3 b	6.8 b	6.8 b	3.4 b
B. spicifera	1	22.3 a,z	18.3 abc,z	8.0 c,z	21.7 ab,y	5.3 d,z
	2	17.7 a,z	11.0 ab,z	9.7 ab,z	5.7 b,z	2.0 b,z
	3	33.3 a,y	16.0 a,z	4.0 c,z	24.3 ab,y	4.0 c,z
B. hawaiiensis	Combined	99.1 a	32.0 b	1.2 b	1.0 b	0.6 b
B. sorokiniana	Combined	45.4 a	18.8 c	19.3 c	31.1 b	2.1 d
B. cynodontis	Combined	20.7 a	17.3 a	1.3 b	0.2 b	0.7 b
B. stenospila	1	58.0 a,y	35.0 b,y	5.7 c,z	11.7 c,z	1.3 c,z
	2	9.3 a,z	10.0 a,z	10.0 a,z	8.0 a,z	0.7 a,z
	3	12.3 a,z	11.3 a,z	7.3 a,z	22.7 a,z	1.7 a,z
D. dictyoides	Combined	0.9 a	0.7 a	0.0 b	0.0 b	0.0 b
E. rostratum	Combined	55.0 a	11.8 b	3.2 b	7.7 b	4.2 b

[a]IC = index card, FP = filter paper, CC = cheesecloth, CF = cotton fabric.
[b]Values are means of three replicates for individual isolates or nine replicates for combined isolates from one of two experiments. Data are presented for individual isolates when isolate × substrate interactions were significant (P = 0.05). Means within rows not followed by the same letter (a–d), and means within columns for each species not followed by the same letter (x–z), differ significantly at P = 0.05 according to Fischer's protected LSD test.

combined isolates on the different substrates. Sporulation on one or more cellulose substrates was significantly greater than on agar in 16 of 23 species or isolate comparisons. Greatest sporulation was observed on IC, FP, CC, and CF in 6, 1, 4, and 5 of these instances, respectively.

In both experiments, high variability in sporulation, with 10-fold or greater differences in numbers of spores formed on individual replicates of treatments, was sometimes observed on the cellulose substrates.

Sporulation on CMA (+/−) filter paper overlay

In preliminary experiments, when whole colonies in 9.5 cm plates were overlaid with IC and FP to enhance sporulation, discs of IC often warped upward, dried out, and did not become completely colonized. Therefore, FP was used as the cellulose substrate to overlay whole colonies because this remained appressed to agar, did not dry out, and was completely colonized by all fungi.

Mean concentrations of spores observed in aqueous suspensions of the nine species from plates of CMA with and without FP overlay are presented in Table 2. For *C. lunata* and *B. sorokiniana*, results of repeated experiments are presented individually due to significant (P = 0.05) heterogeneity of variance. For the remaining seven species, results of repeated experiments are combined.

For all species except *C. geniculata*, numbers of spores observed from CMA overlaid with FP were significantly (P = 0.05) greater than from CMA alone in one or both individual experiments or in combined experiments. For fungi in which significant differences occurred between treatments, numbers of spores observed from CMA overlaid with FP were 2.2–18.3 times greater than numbers from CMA alone (Table 2). However, on CMA both unamended and overlaid with FP, sporulation often occurred in sectors, and FP overlaid on agar was seldom uniformly covered with spores. The magnitude of increased sporulation on FP in these plates was strongly influenced by the amount of sectoring in colonies that grew in them.

Sporulation on soil dilution plates overlaid with filter paper

Samples of soil with bermudagrass sod with attached soil were collected to a depth of 15 cm from a swine waste application site where all nine fungi were present [5, 7], potted, and grown in the greenhouse for 9 months. Soil then was assayed for dematiaceous hyphomycetes by overlaying filter paper onto soil dilution plates. Three assay plates that each received 0.1 or 0.25 ml of a

Table 2. Mean number of spores observed in aqueous suspensions from nine species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* grown in plates of cornmeal agar unamended and overlaid with filter paper

Fungal species	Experiment	Mean number of spores $\times 10^3$ per ml aqueous suspension[a]	
		CMA	CMA with filter paper
C. lunata	1	71.3 b[b]	157.3 a
	2	68.7 a	140.7 a
C. geniculata	Combined	24.8 a	21.4 a
B. spicifera	Combined	8.4 b	81.2 a
B. hawaiiensis	Combined	42.6 b	247.2 a
B. sorokiniana	1	2.0 b	10.1 a
	2	3.7 b	13.0 a
B. cynodontis	Combined	0.2 b	2.8 a
B. stenospila	Combined	0.7 b	13.2 a
D. dictyoides	Combined	< 0.1 b	0.4 a
E. rostratum	Combined	0.2 b	1.0 a

[a]Means based on numbers of spores observed in four hemacytometer counts from each of three replicate plates in which 5 or 10 ml of water was added to the surface with scraping to dislodge spores.
[b]Means within rows not followed by the same letter differ significantly at $P = 0.05$ according to analysis of variance.

10^{-3}, 10^{-4}, or 10^{-5} dilution of soil in water were observed per soil sample. Eleven of 31 soil samples assayed positive for one or more of four species: *C. lunata* (3 samples), *B. spicifera* (7 samples), *B. hawaiiensis* (1 sample), and *E. rostratum* (2 samples). Not more than one colony of each species was observed per sample. The maximal soil population level calculated for observed fungi by these assays was 1.3×10^3 colony-forming units per gram of soil. Whenever sporulation was observed on FP, numerous spores usually were present, and these were often visible macroscopically as dark colonies on the filter paper. In some instances, more limited sporulation was observed only by microscopic observation at 50× or 100×. Other fungi that sporulated on filter paper overlaid on soil dilution plates included species of *Aspergillus*, *Penicillium*, *Alternaria*, *Phoma*, and *Trichoderma*.

Discussion

Results of this study demonstrate that isolates of nine species of *Bipolaris*, *Curvularia*, *Drechslera*, and *Exserohilum* differ greatly in their capacity to produce conidia on three agar media, and that sporulation by all species is significantly increased by growth on one or more cellulose substrates. Therefore, species of these four genera exhibit sporulation responses on cellulose substrates similar to those described for species of *Pyrenochaeta*, *Macrophomina*, *Monilinia*, and *Fusarium* by other authors [17–21]. For most of the nine species, overlaying plates of agar with FP increased production of conidia up to 18-fold and resulted in concentrations suitable for inoculation of plants and other experimental purposes. For four of the species, overlay of soil dilution plates with FP also enabled their detection from a naturally infested soil and estimation of soil population levels. Whether propagules of the other five species known to occur on grasses at this site were present in soil and not detected, or not present in soil, is not known.

On two natural and one synthetic agar medium, species and isolates differed greatly in production of conidia, and small-spore species (*C. lunata*, *C. geniculata*, *B. hawaiiensis*, *B. spicifera*) sporulated more heavily than large-spore species. *Curvularia lunata* always produced the most spores, and this species likely would produce sufficient spores on unamended agar for most experimental purposes. Most other species produced fewer spores on unamended agar, and quantities likely would have been inadequate for inoculation of plants or other purposes. Deficiencies in spore production on agar were especially acute for the five large-spored species, among which only *B. sorokiniana* produced even slight to moderate numbers.

Results of experiments in which sporulation by the nine species was evaluated on four cellulose

substrates clearly indicates that cellulose enhancement of sporulation is a widespread phenomenon among these fungi. In 15 of 18 repeated experiments with the nine species, variation due to substrates was significant at $P = 0.01$, and in the remaining three experiments it was significant at $P = 0.05$. Sporulation on pieces of index card was most often greater than in agar controls, but this was not always the optimal substrate for all species and isolates. Significant isolate (substrate interactions in 6 of 18 experiments indicated considerable inconsistency in the sporulation responses of individual isolates to individual substrates.

Although specific reasons for stimulation of asexual sporulation by growth of the nine fungi on cellulose-containing substrates are not known, a general reason may be that such substrates mimic the plant tissues upon which these fungi grow and sporulate in nature. Cellulose is the most common organic chemical in the world on account of its universal presence as a structural carbohydrate in plant cell walls, and it may comprise 50% of all carbon in vegetation [12, 24]. Many plant pathogenic fungi produce cellulases, primarily as inducible enzymes, when inoculated onto plants or grown on cell walls [24]. These are believed to assist in penetration of host cell walls by causing breakdown of cellulose, and they also may provide energy for fungal growth in the form of breakdown products from this common carbohydrate. Possibly breakdown products that result from enzymatic hydrolysis of cellulose [12, 24], in plant tissues or cellulose-containing substrates, also might function as specific nutrients that promote sporulation by fungal pathogens. Possibly production of different cellulase enzymes by fungal species, and even by isolates within species [24], may have contributed to differences in sporulation observed in this study.

For seven of the nine species studied here, results with FP overlaid on agar indicate that relatively high concentrations of spores can be obtained by this procedure which should be suitable for foliar inoculations of plants and other experimental purposes. These results also further validate the broad cellulose enhancement of sporulation because significant, 2 to 18-fold increases were observed for all spp. except *C. geniculata*. With *D. dictyoides*, however, although sporulation was significantly increased on CMA overlaid with FP in combined experiments, this still did not give sufficient conidia for inoculation of plants or other experimental purposes. Further studies are needed to identify more effective substrates or cultural conditions that will promote greater sporulation by this species.

The variation in sporulation observed between fungal species and isolates in this study, and sectoring for sporulation within colonies, are features that occur commonly in many imperfect fungi [25, 26]. Sporulation itself is a complex phenomenon that is controlled both by environment and genetic factors that may vary between isolates [26]. Among the species observed in this study, sporulation on both unamended agar and cellulose substrates was usually greatest in small-spore species such as *C. lunata* and *B. spicifera*, and less in large-spore species such as *B. stenospila* and *E. rostratum*. Sectoring for sporulation and other morphological features, as observed in this study, also occurs commonly or even regularly among many fungi; this may be caused by heterokaryotic nuclei within thalli, extrachromosomal (mitochondrial) inheritance, or the presence of infectious particles within cytoplasm that lead to progressive senescense of cultures [25].

Although species of *Bipolaris* and *Curvularia* have frequently been isolated from soil [1, 2], we are not aware of published estimates of soil population levels except for *B. sorokiniana*. Population levels described for this apparent soilborne pathogen of wheat and small grains are all relatively low (≤ 900 propagules per gram of soil) [1, 13, 14]. The limited results from this study indicate that similar low population levels also may exist for *B. spicifera* and *C. lunata*, the two species most frequently detected from soil in these studies. The maximal population level estimated for these two species was 1.3×10^{-3} propagules per gram of soil. However, it is not known whether the specific procedures used in filter paper overlay of soil dilution plates were optimal for detection of these species.

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